Identification and Characterization of Suppressors for *rng15* Deletion in Fission Yeast Cytokinesis

By

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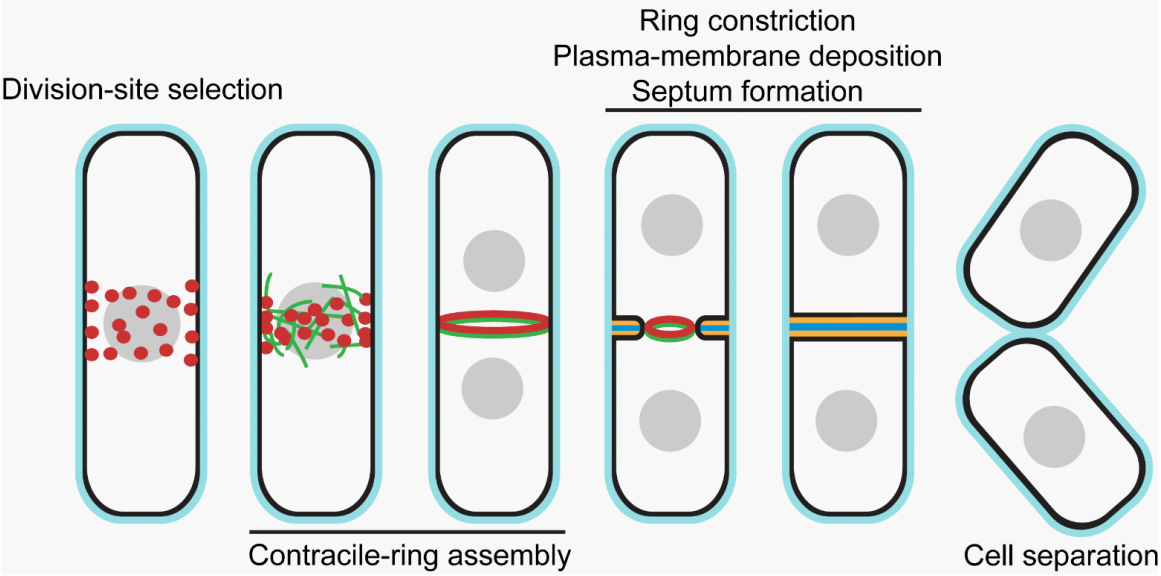
ABSTRACT

While much is known about cytokinesis, the final stage of cell-division cycle, our knowledge on roles of vesicle trafficking during cytokinesis is limited. To further our understanding of exocytosis during cytokinesis, we have investigated the function of Rng15 using fission yeast - a popular model organism for studying cytokinesis. Little is known about Rng15. However, our preliminary studies and its homology with Mso1 from budding yeast, suggest that it participates in exocytosis. Rng15 also shares homology to Mint1, a mammalian adaptor protein involved in exocytosis. Cells with the *rng15* gene deleted (*rng15*Δ) cannot grow at 36°C due to defective cytokinesis. They also accumulate secretory vesicles at the division site. To further investigate how Rng15 functions in cytokinesis, we screened for high-copy suppressors of *rng15*Δ at 36°C by transforming *rng15*Δ cells with plasmid DNA from a fission yeast genomic DNA library. We reasoned that if the proteins being produced from the plasmids rescue the growth of *rng15*Δ cells at 36°C, these proteins are involved in similar pathways as Rng15. So far we have isolated Rng15 and Gmh5 from the screen. Gmh5 is a membrane protein of the Golgi mannosyltransferase complex, which is predicted to be involved in elongation of the polysaccharide mannan backbone and cell wall biogenesis. We will study Gmh5 functions by testing its localization, deletion phenotype, and genetic interactions with *rng15*Δ and other mutations in cytokinesis. In addition, we plan to identify more suppressors with additional screens. Through this investigation, we will have a better understanding of the role of exocytosis in the delivery of materials during fission yeast cytokinesis. Furthermore, we hope to provide others in the field with potential insights into how the Mso1/Rng15 family proteins work in cytokinesis in other systems, including mammals.

INTRODUCTION

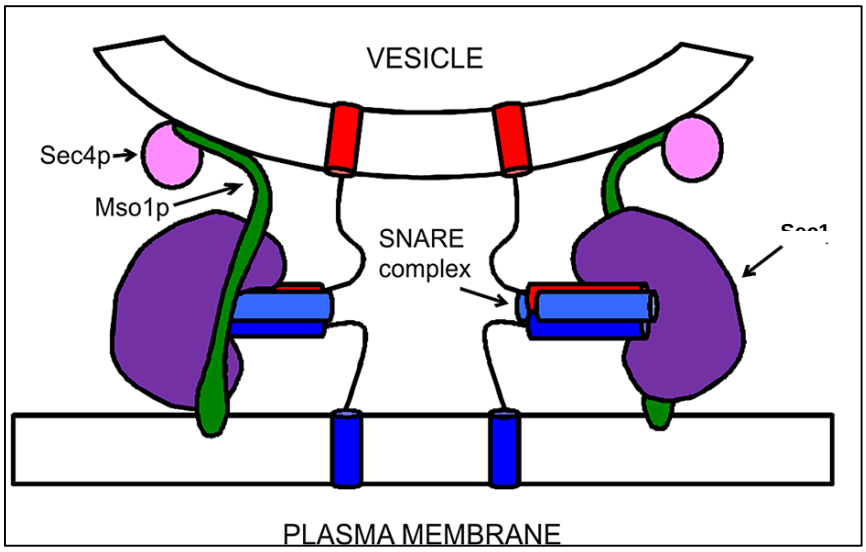
Figure 1



Cytokinesis, or the separation of one mother cell into two daughter cells, is the last step of the cell cycle (Figure 1). The original mother cells splits, resulting in two genetically identical daughter cells. This is a highly conserved mechanism found in nearly all organisms, including yeast and humans. Cytokinesis of fungal and animal cells is a complex event. Four primary steps must be orchestrated so that the cells divide properly (Pollard and Wu, 2010). The division site of the cell is marked and the sister chromatids are separated by the mitotic apparatus. Correct placement of the division site allows for the ultimate creation of two daughter cells, each with its own nucleus. At the selected division site, the cell assembles a contractile ring, a structure made of many proteins and filaments. This ring is attached to the plasma membrane, so when the ring constricts, a cleavage furrow in the plasma membrane is formed. Finally, the plasma membrane is reorganized at the base of the furrow and the two daughter cells are separated. A participant in this division process is a protein called Mso1 (Multicopy Suppressor for Sec One) (Aalto M.,1997) from *Saccharomyces cerevisiae*. When Mso1 is deleted, exocytosis, a process by which the vesicles contents are released through fusion of the vesicle membrane and cell membrane, is inhibited.

Mso1 suppresses different temperature sensitive *sec* gene mutations and mutations in the general components of the secretory pathway (Aalto M. 1997). Mso1 encodes for a small, serine-rich, membrane associated protein. Its N-terminus inserts itself into the lipid bilayer, thus contributing to Mso1's plasma membrane interaction. Its C-terminus binds electrostatically to intracellular membranes (Weber-Boyvat M., 2013).

Figure 2



This activity is essential for sporulation of *S. cerevisiae* (Knop, 2005). Mso1 functions in the terminal stage of secretion by docking the secretory vesicles to the plasma membrane. The loss of Mso1 leads to accumulation of secretory vesicles at the division site, preventing cell division by impairing its terminal step. It is speculated that Mso1 interacts with Sec1 and other components of the vesicle docking complex, particularly the SNARE complex (Weber-Boyvat M., 2013). SNARE complexes are critical for membrane fusion and their assembly requires SM proteins in vivo (Baker et al, 2015), such as Sec1, an SM family protein. Still, Mso1 provides several binding affinities for the SNARE complex, allowing the SNARE complex to participate in cell division.

In *Schizosaccharomyces pombe*, a protein called Rng15 has been shown to be homologous with Mso1 (Knop M. et al, 2005). Little is known about Rng15, though we speculate it, like Mso1, participates in exocytosis due to the high level of evolutionary conservation of this process. Mint1, a mammalian adaptor protein involved in exocytosis, is also homologous to Mso1, leading us to hypothesize that Rng15 potentially plays a role in mammalian cell division. When *rng15* is deleted, cells do not grow at 36°C. In this study we show that Rng15 is a novel membrane-interacting protein with homologs that give insight into its function. Our results show that Rng15 has properties similar to that of Gmh5 in *S. pombe* cytokinesis and vesicle trafficking. In order to determine this, we performed suppressor screens involving *rng15*Δ cells transformed with plasmid DNA from a genome library. This paper will describe the steps taken to determine which genes

rescue the *rng15*Δ phenotype seen in *S. pombe*. The results of these experiments suggest that Rng15 is homologous with Mso1 and Mint1.

MATERIALS AND METHODS

Suppressor Screen

To investigate the role of Rng15 in *S. pombe* cytokinesis, *rng15*Δ cells were obtained from the Wu lab *S. pombe* strain bank. We performed a suppression screen on these cells using plasmid DNA from a genomic library. This genomic library consists of ~ 10 Kb *S. pombe* DNA sequences, which contain on average ~ 3 genes. The transformed *rng15*Δ cells were first grown on YE5S plates at 25°C for two to three days, or were grown until sufficient growth was seen. The resulting colonies were replica plated onto EMM5S-Ura selection plates and grown at 36°C for an additional three days. Plasmid DNA was then extracted from any colonies that grew under these conditions. These rescued plasmids were used to transform *E. coli* cells via electroporation. These *E. coli* cells were grown at 25°C on EMM5S-Ura plates. Once a sufficient amount of cells had grown, we extracted these plasmids and sent them for sequencing. By doing this screen, we discovered that Gmh5 rescued the *rng15*Δ phenotype.

Synthetic Lethality Test

Once Gmh5 had been identified, we crossed a *gmh5*Δ strain with a *rng15*Δ strain, creating a hybrid strain that had both the *gmh5* and the *rng15* genes deleted. We grew the resulting strain on YE5S plates at 25°C for two to three days or until sufficient growth was seen.

RESULTS

Suppression Screen

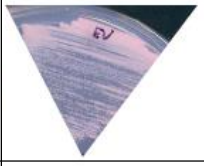
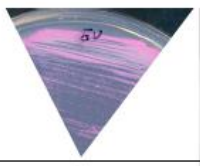
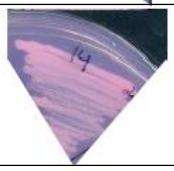
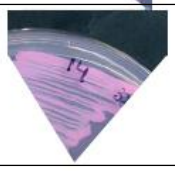
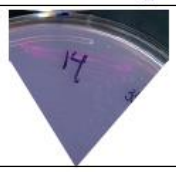
5 screens were performed. In each screen ~ 3-4,000 colonies were analyzed. Thus, 15-18,000 colonies were screened in total. Since there are approximately 5,000 genes currently known in the *S. pombe* genome, we can assume that we sufficiently covered the *S. pombe* genome with our screens. Three genes were identified that rescued the *rng15*Δ phenotype (Table 1).

Table 1

Suppressor Candidate	Gene	Function
9, 10, 15, 19, 22, 24	Rng15	Unknown
16	Cob1	Electron Transport
14	Gmh5	Cell Wall Biogenesis

Table 2

Growth phenotypes of supressors

	25°C	32°C	36°C
Empty Vector			
Supressor 15			
Supressor 16			
Supressor 14			

Our suppression screen findings are summarized in Tables 1 and 2. We used an empty vector as our control. As seen in Table 2, when no gene is inserted in place of *rng15*, the cells grew well at 25°C and 32°C, but did not grow at 36°C. This control shows that the genes that did become

inserted genuinely suppressed the *rng15⊿* phenotype. In most candidates, the transformation reinserted the *rng15* gene into the *rng15*Δ cells. In Table 2, one suppressor, Suppressor 15, illustrates the phenotype of those cells rescued by *rng15* insertion. It mimics that of the wild type phenotype; that is, growing well at all temperatures. This reconfirms that Rng15 is necessary for cytokinesis, but does not give much insight into Rng15's primary function. In candidate 16, *cob1*, a mitochondrial gene, was shown to rescue the *rng15*Δ phenotype. This was a partial rescue, as those cells rescued by *cob1* grew well at 25°C and 32°C, but grew poorly at 36°C. Cob1 is an electron transporter that is primarily expressed during the vegetative growth phase of *S. pombe*. It is possible that the over expression of Cob1 provided enough energy for the cell to divide, but more research is needed to confirm this. In any case, neither of these genes provided much insight into Rng15's functioning since they do not mimic Mso1 and do not directly relate to cell division. Suppressor candidate 14, however, showed promising results. These cells were rescued by *gmh5*, a gene that aids in cell wall biogenesis. As observed with *cob1*, *gmh5* also partially rescued the *rng15*Δ phenotype.

Synthetic Lethality Test

We grew a *gmh5⊿* strain at various temperatures to see its phenotypes (Figure 3). Overall, *gmh5⊿* cells are viable at all temperatures. However, the *gmh5⊿* cells are heavily lysed in comparison to wild type cells, indicating that even though they proliferate well, the *gmh5⊿* cells are not healthy.

Figure 3

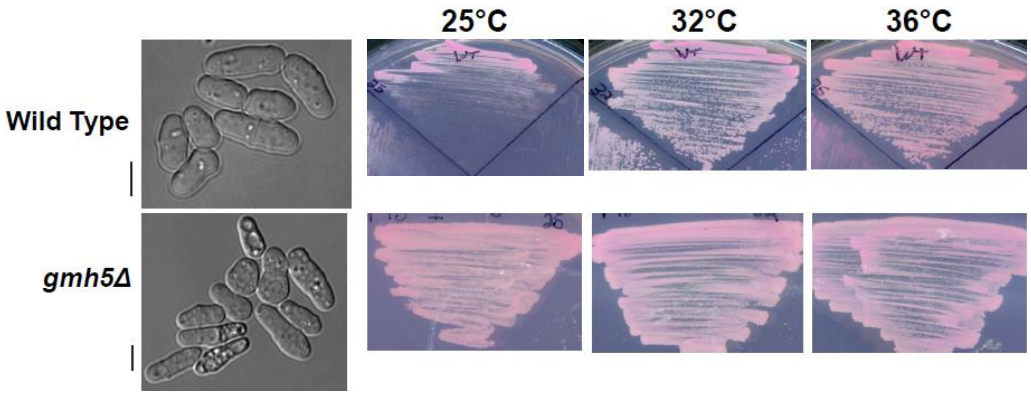
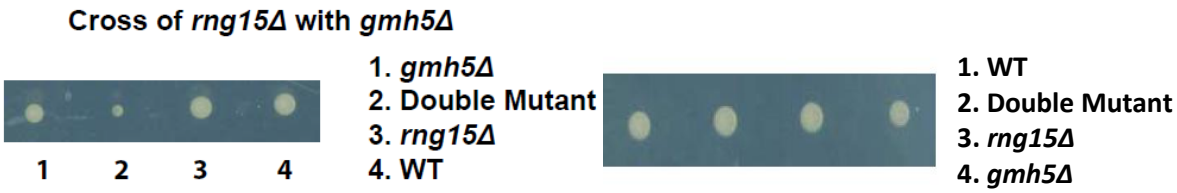


Figure 4

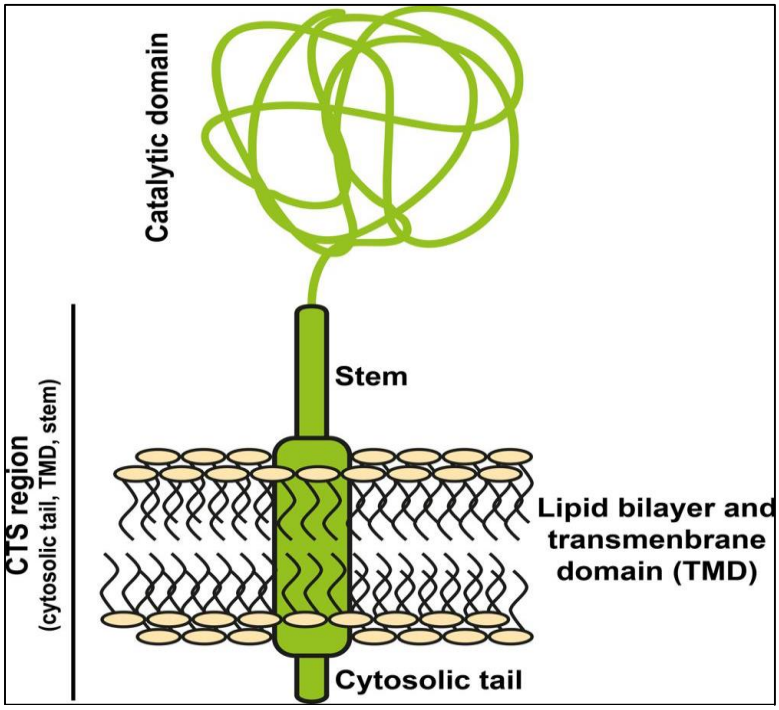


The results of the *gmh5*∠*/rng15*∠ cross are shown in Figure 4. Small but promising growth of the double mutant strain indicates that there is no synthetic lethality between *gmh5* and *rng15*, otherwise there would be no growth at all. This indicates there is no functional relationship between the *gmh5* and *rng15* genes.

DISCUSSION AND FUTURE DIRECTIONS

Discussion

Figure 5



Structure of glycosyltransferases (Loos A. & Steinkellner H., 2014)

Through our research, we have found that Gmh5 rescues the *rng15*Δ phenotype. Gmh5 is a membrane protein of the Golgi mannosyltransferase complex, which is predicted to be involved in elongation of the polysaccharide mannan backbone and cell wall biogenesis. In yeast, the Golgi apparatus interacts with the necessary cytosolic components for transport vesicle formation and consumption and contains several mannosyltransferases to extend N-glycans with mannose. This is a highly conserved network, involving multiple galactosyltransferase activities along the secretory pathway in order for proper division to occur. The Gmh5 protein is a Type II transmembrane protein responsible for targeting the membrane to the correct compartment of the Golgi apparatus (Essl et al., 1999). Gmh5's final glycoprotein contains a single membrane spanning domain near the N-terminus, producing a transmembrane arrangement identical to that of the mammalian glycosyltransferases shown in Figure 5 (Chappell T. G et al., 1994). Since the over expression of Gmh5 rescued the *rng15*Δ phenotype, we speculate that the glycosyltransferases aided in the formation of the cell walls, allowing the cells to divide.

Our results initially confirm that Rng15 is necessary for cell growth at 36°C, with many *rng15⊿* colonies being rescued by *rng15*. Also revealed by the suppressor screen was that Cob1 is a potential suppressor for the *rng15⊿* phenotype. While this was not the primary focus of our study, it is important to note that Cob1 rescues cell division at high temperatures. The main finding of our study was that *gmh5* is a potential suppressor for the *rng15⊿* growth defect at 36°C. This is potentially due to *gmh5* overexpression aiding in the creation of cell wall in *rng15's* absence. We also see that there are no synthetic effects between *gmh5* and *rng15*. This suggests that while cells can grow in the absence of *rng15* and *gmh5*, they are not essential for cell division at 25°C. The fact that the resulting *rng15⊿/gmh5⊿* were not healthy indicates that perhaps *rng15* and *gmh5* are necessary for cellular maintenance.

Future Directions

Gmh5's exact role in cytokinesis and exocytosis is not currently known, so more research is needed to confirm our speculations about its functions. Overexpression of Gmh5 in *rng15⊿* cells could give provide this insight. Gmh5's localization and function during cell division should also be investigated in order to determine its exact role in the overall process. A recent paper did a high throughput screen for *S. pombe* (Ryan C.J., 2012). It implicates that Gmh5 may be involved in hundreds of genetic interactions, which could potentially effect cell division. Further research into these specific interactions and potential pathways is needed to confirm Gmh5 and Rng15's role in alternate pathways and other mammals. The exact phenotype of the *gmh5⊿/rng15⊿* cells should be determined. Additional screens should be performed to identify other suppressors. More research is needed to investigate Cob1's role in cytokinesis and exocytosis.

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